2. Research Reports

2.1. Soil Biological Fingerprints from Meadow Steppe and Steppe Communities with Native and Non-Native Vegetation

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2.1.1. Introduction

All cells contain fatty acids that can be extracted and esterified with methanol to form fatty acid methyl esters (FAMEs) (Klug and Tiedje 1993; Kennedy 1994). When the FAMEs extracted from a soil sample are analyzed using gas chromatography, the resulting profile of fatty acids constitutes a "fingerprint" of the organisms in the sample. Soil microbial communities can be compared by extracting FAMEs from soil and using multivariate statistical techniques on the resulting FAME profiles to analyze any differences.

Different soil microorganisms are associated with the roots of different plant species (Westover et al. 1997). The soil biological fingerprints associated with non-native plants may differ from those associated with natives, either because alien species colonize sites with particular soil microbiota, or because the microbial community changes as a result of colonization by exotics. In this study, we compared the biological fingerprints of surface soils from meadow steppe and steppe communities in Palouse grasslands dominated by non-native and native vascular plants.

2.1.2. Methods

Study sites

We obtained data on vegetation, cryptogams, and soil biota at two meadow steppe sites, Kramer and Paradise Ridge, and two steppe sites, Waha and Colfax (Figure 1). Kramer and Paradise Ridge support Idaho fescue/common snowberry (*Festuca idahoensis/Symphoricarpos albus*) associations, while Waha and Colfax support bluebunch wheatgrass/Sandberg's bluegrass (*Pseudoroegneria spicata* spp. *spicata* [=*Agropyron spicatum*]-*Poa secunda*) associations (Weddell and Lichthardt 1998). At meadow steppe sites, native perennial grasses and low to medium shrubs are accompanied by a well developed forb component, whereas at steppe, perennial bunchgrasses are the sole dominants (Daubenmire 1970). The major non-native plants present were bulbous bluegrass (*Poa bulbosa*), hairy vetch (*Vicia villosa*), and alien annual bromes (*Bromus* spp.) at Colfax; yellow star-thistle (*Centaurea solstitialis*), Kentucky bluegrass (*Poa pratensis*), piedmont bedstraw (*Cruciata pedemontana*), and annual grasses including medusahead (*Taeniatherum caput-medusae*), ventenata (*Ventenata dubia*), and annual

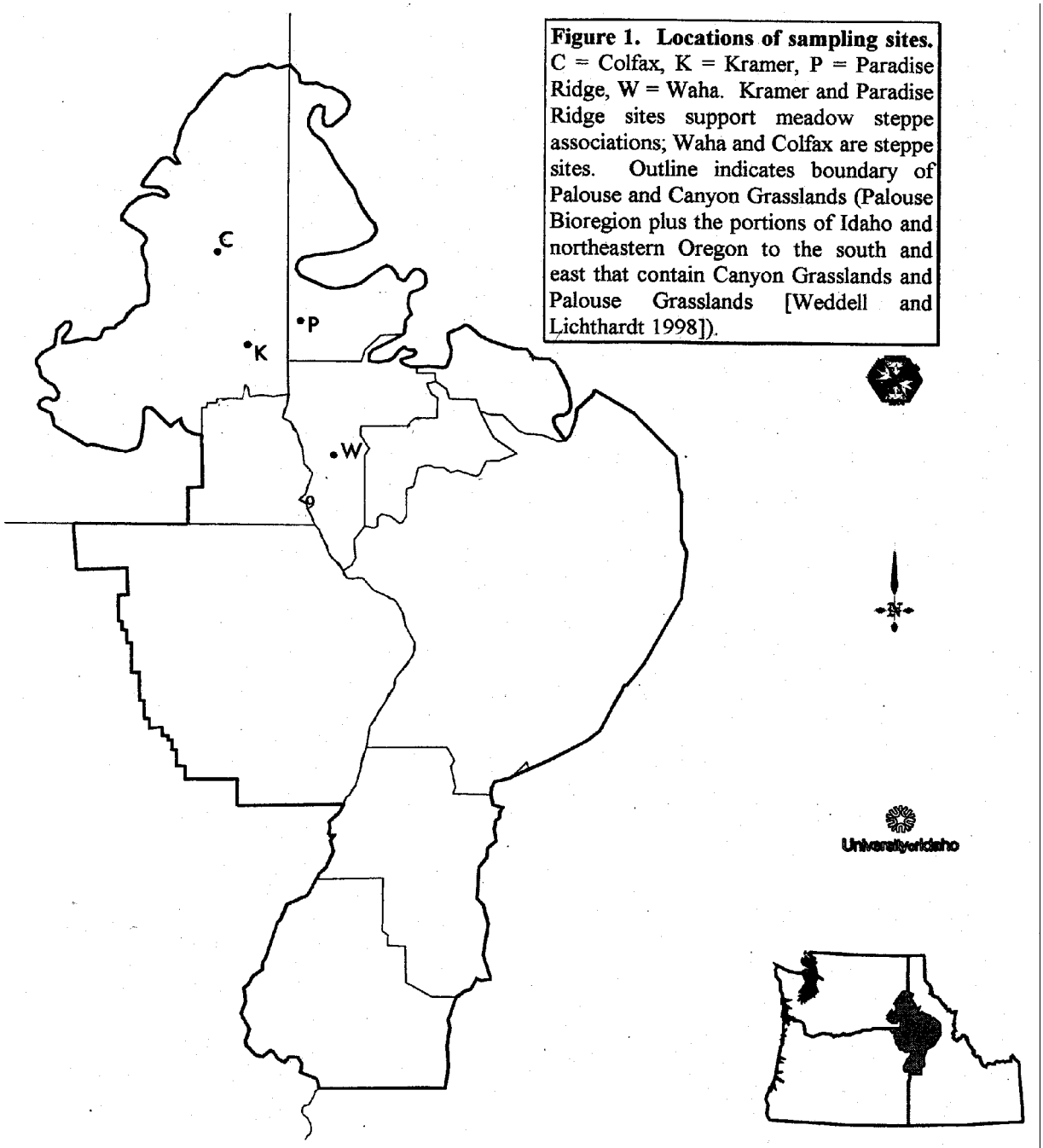


Figure 1. Locations of sampling sites. C = Colfax, K = Kramer, P = Paradise Ridge, W = Waha. Kramer and Paradise Ridge sites support meadow steppe associations; Waha and Colfax are steppe sites. Outline indicates boundary of Palouse and Canyon Grasslands (Palouse Bioregion plus the portions of Idaho and northeastern Oregon to the south and east that contain Canyon Grasslands and Palouse Grasslands [Weddell and Lichthardt 1998]).

bromes at Waha; cheatgrass (*Bromus tectorum*) at Paradise Ridge; and Kentucky bluegrass at Kramer.

Collection of field data and samples

Soil samples and data on vegetation at the four study sites were collected between May 22 and June 4, 1999. At each study site, one 20-m transect was set up in vegetation dominated primarily by native plants, and a similar transect was established nearby in vegetation dominated primarily by non-native species. At Kramer, Waha, and Colfax, the transects were the same as those used in 1998 to monitor threats to native vegetation (Weddell and Lichthardt 1998; transect 73 at Kramer). Vegetation was sampled using a 20-x-50-cm plot frame placed at 0.5-m intervals along each transect, and the canopy coverages of all species of vascular plants except annual grasses were recorded using six coverage classes (0-5%, 5-25%, 25-50%, 50-75%, 75-95%, and 95-100%). Mean canopy coverage values for all species of vascular plants, except annual grasses, was computed using the midpoints of the coverage classes, and coverage values were summed by life form for exotic and native species (i.e., native perennial graminoids, alien perennial graminoids, native perennial forbs, etc.). The canopy coverage of native annual grasses as a group was also estimated; as was the coverage of alien annual grasses. The coverages of rocks, litter, and bare ground were estimated in the same way. Soil temperature at a depth of 7 cm was measured at 5, 10, and 15 m along the transects. Soil stoniness was estimated using a sharpened piece of rebar 9 mm in diameter with a cross-bar at the top. The rod was thrust into the soil at right angles to the surface at 0, 5, 10, 15, and 20 m along the transects, and the depth of penetration until a rock fragment or bedrock stopped the bar was measured. Soil strength was measured with a penetrometer at 1-m intervals along each transect. Soil samples were collected 5, 10, and 15 m along the transects and kept on ice until they were transported to the laboratory.

Fatty acid extraction

FAMEs were extracted from two 1-g subsamples from each soil sample using the procedure described by Kennedy and Busacca (1995). We used the Sherlock Microbial Identification System protocol (MIDI Labs, Inc., Newark, DE), with the addition of an internal standard (nonadecanoic acid methyl ester). Samples were hydrolyzed with sodium hydroxide in aqueous methanol. To hydrolyze the samples, 1 mL of a solution formed from 45 g NaOH in 150 mL methanol and 150 mL deionized water was added, and the samples were heated for 30 min in a 100°C water bath. After the samples were cooled, the fatty acids were methylated by adding 2 mL 6.0 N HCl in aqueous methanol (325 mL 6.0 N HCl in 275 mL methanol). FAMEs were extracted from the aqueous phase to an organic phase with 1 mL hexane:methyl-tert-butyl-ether (1:1 volume:volume). One hundred L of nonadecanoic acid methyl ester (161 M) in hexane:methyl-tert-butyl-ether (1:1 volume:volume) were added, and the samples were placed on an end-over-end mixer for 10 min. Samples were centrifuged at 3,000 rpm for 2 min, and the organic phase was transferred to an acid-washed tube. The extraction process was then repeated without the internal standard. The combined organic phases were base-washed by the addition 3

mL of 1.2% NaOH in deionized water with 5 min of end-over-end rotation. Samples were allowed to evaporate over night, after which they were resuspended in 150 L of hexane:methyl-tert-butyl-ether.

Gas chromatography of fatty acids

Samples were analyzed by a 5890A Hewlett Packard gas chromatograph with a flame ionization detector, HP-IB communications, and HP 3365 ChemStation software, using the Eukary method of the Sherlock Microbial Identification System (Sasser 1990). ChemStation operated the sampling, analysis, and integration of the samples under Eukary method parameters. The temperature was ramped at 5 ºC/min from 170ºC-300ºC, where it remained until the end of the 38-min run. A Hewlett Packard Ultra-2 nonpolar fused silica capillary column (25 m by 0.20 mm by 0.33 m) was used. Hydrogen was the carrier gas, nitrogen was the make up gas, and air was used to support the flame at a flow rate of 30, 30, and 400 mL/min.

Statistical analysis

We used a pattern recognition program to identify differences and similarities between the fatty acid fingerprints of the different subsamples (Sasser 1990). The data for FAMEs with up to 20 carbons were analyzed using principal component analysis (PCA) in SAS (SAS Institute 1988). We limited our analysis to FAMEs with less than 21 carbons, since these are the dominant fatty acids in bacteria (Haack et al. 1994). PCA is a type of multivariate analysis that expresses the similarities and differences of data sets in terms of a small number of principal components. The variance-covariance structure of a data set is explained through a few linear combinations of the original variances, with coefficients equal to the eigenvectors of the correlation matrix (Jolliffe 1986). The statistical component that gave the greatest separation of the groups was designated PRIN1, the component that gave the next greatest separation was designated PRIN2, and so on (Kennedy and Busacca 1995).

2.1.3. Results

Characteristics of the vegetation and soils at the four study areas are summarized in Table 1, Figure 2 and Figure 3. The steppe sites (Waha and Colfax) tended to have higher coverage of perennial graminoids than the meadow steppe sites (Kramer and Paradise Ridge) (Figure 2). Cryptogam cover tended to be higher at the steppe sites than at the meadow steppe sites, and higher in the native vegetation at those sites than in the non-native vegetation (Figure 3). At the steppe sites, the non-native plant transects were generally less rocky (as evidenced by lower coverage of surface rock and deeper penetration of the test bar) than the native plant transects (Table 1).

	Kramer		Paradise Ridge		Waha		Colfax	
	Native	*Non-native*	Native	*Non-native*	Native	*Non-native*	Native	*Non-native*
Plant community type	Meadow steppe		Meadow steppe		Steppe		Steppe	
Vegetation								
Shrubs	13.4	*18.6*	30.3	*10.5*	0.0	*0.0*	0.0	*0.0*
Perennials								
Graminoids								
Native perennial graminoids	21.0	*17.4*	15.4	*18.6*	54.4	*5.9*	29.8	*7.9*
Alien perennial graminoids	**19.8**	***3.3***	**0.0**	***0.0***	**0.0**	***6.9***	**17.9**	***9.4***
Forbs								
Native perennial forbs	87.4	*72.8*	23.5	*24.1*	37.1	*31.8*	9.3	*0.4*
Alien perennial forbs	**0.3**	***0.0***	**0.0**	***0.0***	**0.0**	***0.0***	**0.0**	***0.0***
Coverage native perennial forbs and graminoids	108.4	*90.1*	38.9	*42.8*	91.5	*37.6*	39.0	*8.3*
Percent native perennial graminoids	12.7	*10.2*	15.2	*21.5*	46.6	*6.5*	44.8	*7.9*
Annuals/biennials								
Graminoids								
Native annual grasses	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	0.6	*0.0*
Alien annual grasses	**1.8**	***13.7***	**6.9**	***7.8***	**16.9**	***25.6***	**2.0**	***33.5***
Forbs								
Native annual/biennial forbs	14.5	*24.1*	21.1	*17.1*	3.6	*2.3*	0.4	*13.0*
Alien annual/biennial forbs	**7.0**	***21.0***	**4.3**	***8.5***	**4.8**	***17.5***	**6.5**	***35.4***
Total vascular canopy coverage	165.0	*170.8*	101.4	*86.6*	116.8	*89.9*	66.4	*99.5*
Total coverage exotics	28.8	*37.9*	11.1	*16.3*	21.6	*50.0*	26.4	*78.3*
Percent exotic coverage	16.8	*22.2*	11.0	*18.8*	18.5	*55.6*	*39.7*	*78.6*
Total coverage annuals	23.3	*58.8*	32.3	*33.4*	25.3	*45.4*	9.5	*81.9*
Percent annual coverage	14.1	*34.4*	31.8	*38.5*	21.6	*50.5*	14.3	*82.3*
Total exotic annuals	8.8	*34.7*	11.1	*16.3*	21.6	*43.1*	8.5	*68.9*
Percent exotic annuals	5.3	*20.3*	11.0	*18.8*	18.5	*48.0*	12.8	*69.2*
Mosses	14.5	*1.0*	1.1	*7.1*	25.9	*14.0*	12.9	*0.0*
Lichens	0.1	*0.0*	0.5	*3.3*	1.4	*0.0*	6.4	*0.0*
Total cryptogam coverage	14.6	*1.0*	1.6	*10.4*	27.3	*14.0*	19.3	*0.0*
Bare ground	5.6	*11.0*	17.5	*22.0*	2.4	*6.0*	16.4	*11.3*
Rock	0.0	*0.0*	3.6	*5.5*	15.0	*0.5*	18.9	*5.9*
Litter	76.9	*52.1*	54.8	*56.5*	52.3	*91.3*	34.5	*68.4*
Soils								
Soil strength (lbs per sq. inch)	0.49	*0.19*	0.10	*0.16*	0.21	*0.17*	1.58	*1.75*
Soil temperature (degrees C)	17.7	*18.3*	13.7	*12.3*	19.7	*15.7*	36.7	*31.0*
Soil stoniness (cm to rock)	54.0	*45.8*	23.4	*23.6*	13.6	*36.4*	5.8	*24.6*
Slope (%)	11	*13*	25	*23*	9	*11*	22	*24*
Aspect	NW	*WSW*	ENE	*ENE*	SW	*SW*	E	*E*
Elevation (m)	850	*850*	1,070	*1,070*	1,100	*1,100*	670	*670*
Soil type	Palouse-Thatuna silt loam		Schumacher variant loam		Mallory-Jacket complex		Gwin-Linville complex	

Table 1. Characteristics of vegetation and soils at two meadow steppe sites (Kramer and Paradise Ridge) and two steppe sites Waha and Colfax).

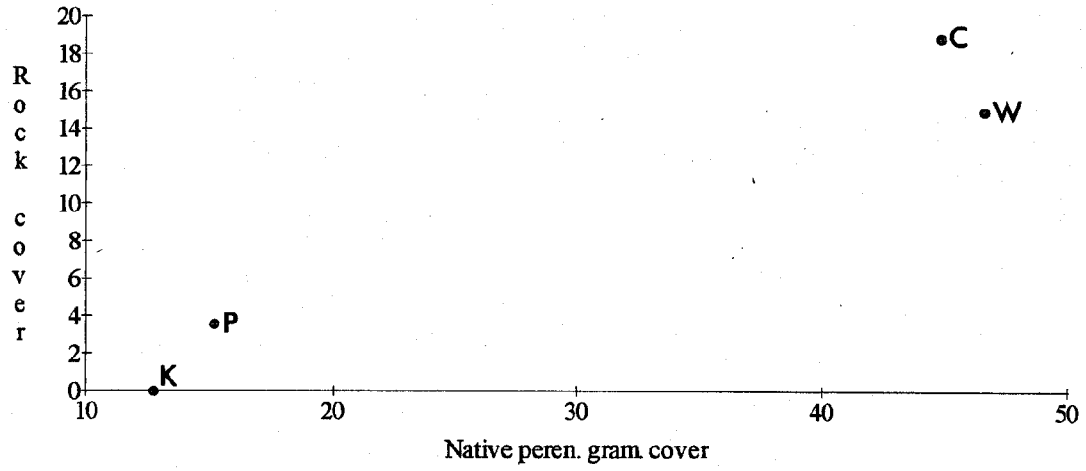


Figure 2. Rock cover and native perennial graminoid cover of sampling sites. Only data for transects dominated by native vegetation are shown. C = Colfax, K = Kramer, P = Paradise Ridge, W = Waha.

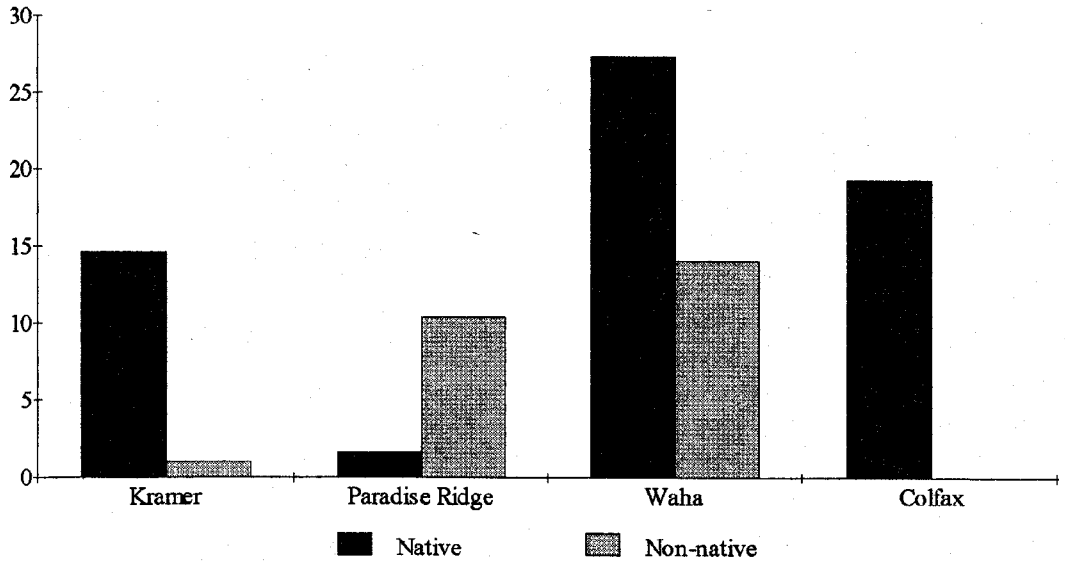


Figure 3. Comparison of coverage of mosses and lichens along transects dominated by native and non-native vegetation at four study sites. Waha and Colfax are steppe sites; Kramer and Paradise Ridge are in meadow steppe.

We analyzed 12 subsamples (2 subsamples/sample, 3 samples/transect, 2 transects/site) from each site except Colfax. We encountered technical difficulties with two of the Colfax subsamples, so at that site we had only 10 subsamples. Figure 4 shows an example of a gas chromatogram fatty acid fingerprint for one of the subsamples.

The distribution of the subsamples according to the first two principal components is plotted in Figure 5. The first principal component (PRIN1) explained 15.6% of the variance, and the second (PRIN2) accounted for 11.8% of the variance in the subsamples. Plant association seemed to have more effect on soil microbiota than the degree of coverage by non-native species. The profiles from bluebunch wheatgrass-Sandberg's bluegrass associations at Waha and Colfax (shown as circles on Figure 5)—tended to occupy high positions along the PRIN1 axis and low positions along the PRIN2 axis. The reverse was true for the profiles from meadow steppe vegetation—Idaho fescue/common snowberry associations at Paradise Ridge and Kramer—(shown as triangles in Figure 5); they scored relatively high on PRIN2 and low on PRIN1.

The separation among FAME profiles from transects dominated by native and non-native vegetation (solid and open symbols respectively) was less dramatic, although some differences were apparent. Samples taken in native vegetation (solid symbols) tended to have a narrower distribution along PRIN2 than samples from non-native vegetation (open symbols).

2.1.4. Discussion

Using profiles of fatty acid methyl esters extracted from surface soil samples, we found differences between the microbial communities associated with Idaho fescue/common snowberry associations and bluebunch wheatgrass-Sandberg's bluegrass associations. We did not find pronounced differences between the microbiota of stands dominated by non-native and native plants, however, although some differences were apparent. This may be because non-native species—especially annual bromes, such as cheatgrass and ventenata, and, at Kramer, the perennial Kentucky bluegrass—are present even in communities dominated by native grasses and forbs. We did not look at the microbial associations of particular species. Such an analysis might reveal differences in soil microorganisms that were not revealed in this study.

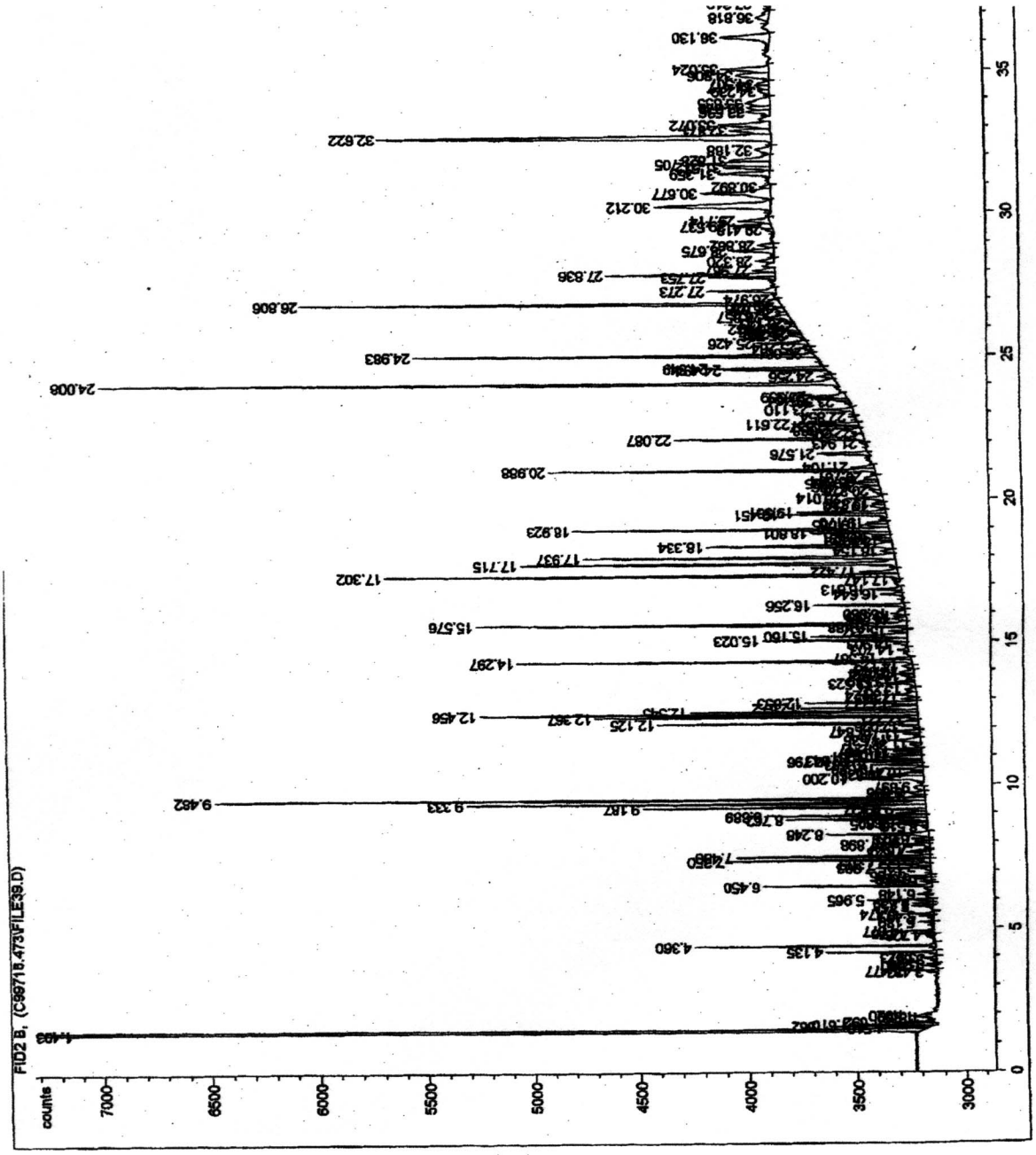
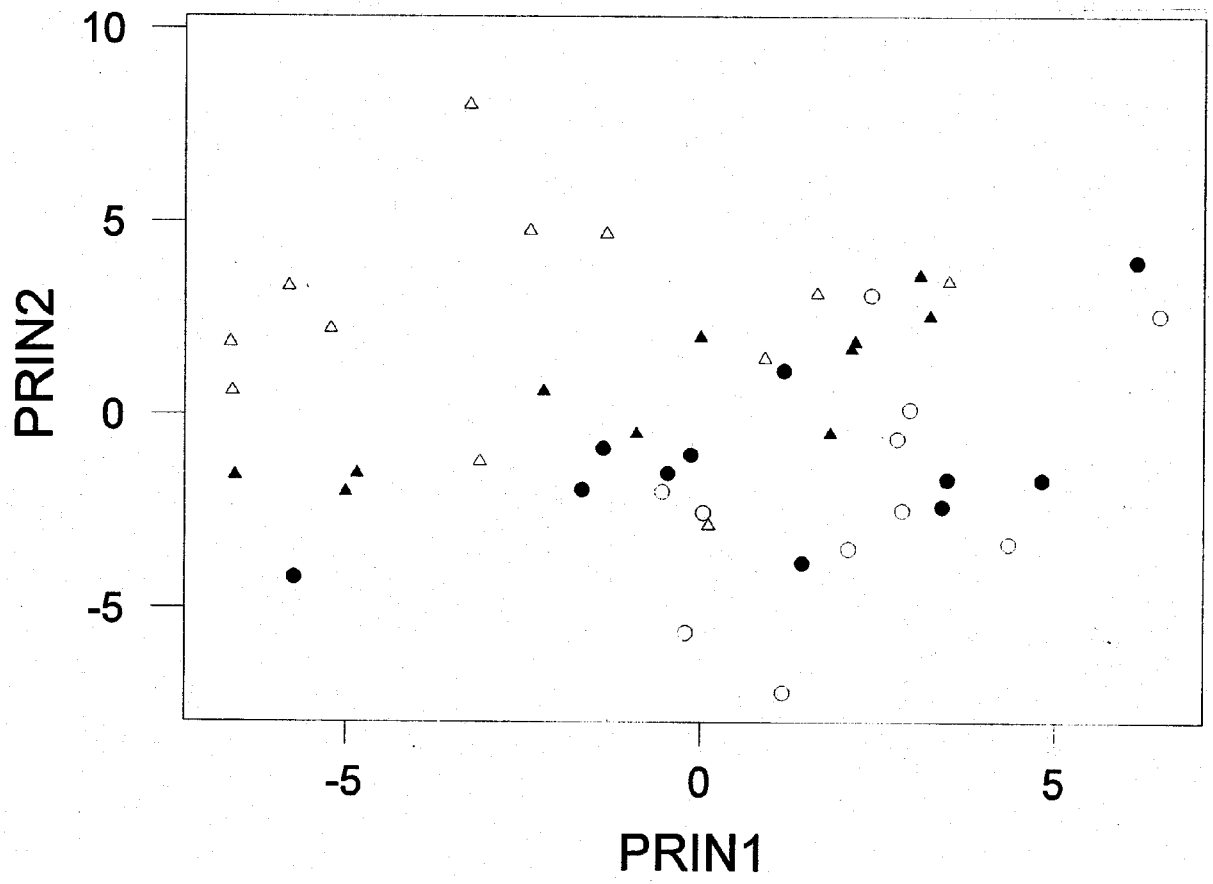


Figure 4. Fatty acid fingerprint of a soil subsample collected along the native transect at the Kramer site.

Figure 5. Two-dimensional plot of the first two principal components derived from profiles of FAMEs extracted from soils dominated by native and non-native vegetation at two meadow steppe and two steppe sites. Open circles: non-native steppe (Waha and Colfax); open triangles: non-native meadow steppe (Kramer and Paradise Ridge). Filled circles: native steppe (Waha and Colfax); filled triangles: native meadow steppe (Kramer and Paradise Ridge). Note that the separation of meadow steppe and steppe samples (circles and triangles) is greater than the separation of native and non-native samples (open and filled symbols).



2.1.5. Literature cited

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